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Effects of 5-lipoxygenase inhibitor zileuton on airway responses to inhaled swine house dust in healthy subjects

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Summary
Background: Inhalation of swine house dust induces acute airway inflammation and increased bronchial responsiveness in healthy subjects.
Objective: The aim of the study was to investigate whether 5-lipoxygenase products such as leukotrienes may have a role in this reaction.
Methods: Twenty-three healthy subjects were randomised into two groups receiving treatment with either zileuton (600 mg) or placebo four times a day. After 5 days of treatment, all subjects were exposed for 3 h in a swine barn. Bronchial responsiveness, exhaled nitric oxide (NO), and mediators in nasal lavage (NAL), blood and urine were measured before and after the exposure.
Results: The exposure induced an increased bronchial responsiveness to methacholine in both groups with 2–3 doubling concentration steps, no significant difference between treatments. Leukotriene E_4 in urine increased significantly following exposure in the placebo group from 37.3 (29.1–45.6) (mean (95% confidence interval)) ng/mmol creatinine to 47.7 (36.3–59.0) ng/mmol creatinine ($P<0.05$), but not in the zileuton group. The post-exposure increase of LTB_4 levels in NAL fluid was totally abolished in the zileuton group ($P<0.05$ vs. the placebo). The levels of exhaled NO increased significantly ($P<0.01$), two-fold in both groups. The PGD_2 metabolite 9α, 11β-PGF_2 increased in placebo-treated subjects ($P<0.01$; $P<0.05$ vs. zileuton), strengthening mast cell participation. Neutrophil counts and levels of IL-6 in peripheral blood increased in both groups, with a significantly larger increase in zileuton treated subjects ($P<0.05$ and $P<0.001$, respectively compared to placebo).

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Conclusions: Pre-treatment with clinically recommended doses of the 5-lipoxygenase inhibitor zileuton did not affect the increase of bronchial reactivity induced by swine dust exposure. The intervention totally abolished the LTB_4 release in NAL fluid, but only partially inhibited the formation of leukotrienes as monitored by urinary levels. The enhanced increase of neutrophils and IL-6 in peripheral blood in the zileuton group, suggests that inhibition of 5-lipoxygenase may have pro-inflammatory effects.

Introduction

A standardised exposure to swine house dust has been well documented to induce pulmonary inflammation associated with a marked increase in bronchial responsiveness to methacholine in healthy, previously unexposed subjects.[1–3] As the inflammatory response has several similarities to the inflammation observed in patients with chronic bronchitis and chronic obstructive pulmonary disease (COPD)[4] as well as the reaction following exposure to tobacco smoke,[5] this particular model may, in addition to its direct bearing on occupational medicine, provide insights into mechanisms of relevance to chronic bronchitis and/or COPD. Thus, the post-exposure increase of bronchial responsiveness is associated with an intense inflammatory response characterised by a prominent neutrophilia and increased levels of cytokines such as interleukin-6 (IL-6), IL-8 and tumour necrosis factor-α (TNF-α) in the upper and lower airways.[6–8] The mechanisms of the increased bronchial responsiveness remain unknown, and the relation between the observed inflammatory response and the changes in bronchial responsiveness is another area of uncertainty. Previous results with increased urinary excretion of the prostaglandin (PG) D_2 metabolite 9α, 11β-PGF_2 have strongly indicated mast cell activation following swine house dust exposure.[3] Exhaled nitric oxide (NO) has been found to be increased in many inflammatory airways diseases such as asthma[13] and chronic bronchitis,[14] and there are experimental data supporting direct interactions between NO and the 5-lipoxygenase (LO) pathway.[15]

We have earlier reported that exposure to swine house dust was associated with increased urinary excretion of leukotriene E_4 (LTE_4) and increased levels of LTB_4 and LTE_4 in nasal lavage (NAL) fluid following exposure.[3] Cysteinyl-leukotrienes (LTC_4, LTD_4 and LTE_4) mediate bronchoconstrictive responses in asthmatics, and drugs which block the formation or the actions of cysteinyl-leukotrienes (cys-LTs) are now used as treatment of asthma.[9] There is also some evidence that anti-leukotriene drugs may attenuate bronchial hyperresponsiveness in asthmatic subjects.[10,11] The 5-lipoxygenase and leukotriene biosynthesis inhibitor zileuton has been shown to have anti-asthmatic effects and is registered in the US for treatment of asthma.[12]

The present intervention study was initiated to evaluate the involvement of leukotrienes in the reactions that follow exposure in a swine house. In view of the prominent neutrophilic response and our earlier findings of increased levels of LTB_4, we attempted to obtain an overall inhibition of the leukotriene pathway by treatment with zileuton (Zyflo®) prior to exposure. Urine was collected before and after exposure for measurements of urinary LTE_4 and the concentrations of LTB_4 and LTE_4 were measured in NAL fluids to determine the drug effect on the leukotriene pathway. We also investigated mast cell activation by measurements of the mast cell marker 9α, 11β-PGF_2. Influences on cytokine and cell response in blood and NAL fluid were monitored. The bronchial reactivity and the levels of exhaled NO were measured before and after exposure to evaluate any relation between the 5-lipoxygenase inhibition, bronchial responsiveness and NO in the response to swine house dust.

Material and methods

Subjects

Twenty-four (12 F/12 M) healthy, non-smoking subjects, with no history of allergic diseases, participated in the study. Mean age was 28 (range 20–47) years in the placebo group and 26 (range 21–41) years in the zileuton group. All subjects were previously unexposed or only occasionally exposed to farming environment and all participants gave their informed consent. The study was approved by the local ethics committee at Karolinska Institutet (Dnr 99-463).

Study design

The study design is described in Fig. 1. At the first visit, lung function and exhaled NO were measured,

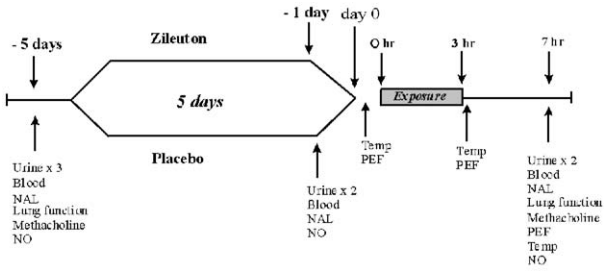

Figure 1 Study design. Twenty-three healthy subjects treated with zileuton ($n = 11$) or placebo ($n = 12$) for 5 days prior to exposure in a swine house. NAL = nasal lavage, NO = nitric oxide, PEF = peak expiratory flow.

a bronchial methacholine provocation test and a NAL were performed. In addition, samples of blood and urine were collected. At this occasion the subjects were also randomised to treatment with either zileuton (600 mg) or equivalent placebo capsules (qid) in a single blind manner. Twelve subjects received placebo (4 F/8 M) and 12 received zileuton (8 F/4 M). One subject (F) allocated to the zileuton group decided to withdraw from the study during the first day of treatment due to possible drug-related urticaria.

Medications were continued for 5 days. After 4 days of medication subjects returned for blood and urine sampling, another NAL and measurement of exhaled NO, to test if basal levels were influenced by the drug. The following day, the subjects were exposed for 3 h while weighing pigs in a swine confinement building holding about 300 pigs. The subjects were instructed by a swine farmer to shove the pigs into a weighing box. This is a quite strenuous working situation associated with high exposure levels to swine house dust. Six subjects, three from each group, were exposed at each occasion. Personal samplers were used to monitor exposure levels throughout the whole exposure period. Pre- and post-exposure, peak expiratory flow (PEF) and body temperature were measured and urine samples were collected. The measurements and urine sampling were repeated approximately every second hour up to 5–6 h after the exposure. The last medication capsule was taken immediately after completion of the exposure.

Seven hours after the start of the exposure, NAL, blood sampling, measurements of exhaled NO, lung function and bronchial methacholine provocation test were performed.

Exposure measurements

IOM filter cassettes (25 mm) (SKC LTD, Dorset, England) and plastic cyclones (25 mm) (Casella LTD, London, England) were used to monitor inhalable and respiratory dust levels, respectively. Samplers were placed in the breathing zone on subjects carrying personal samplers. Three to four samplers were used for measurements of inhalable and respirable dust levels, respectively. An average value from each exposure occasion were calculated. The IOM cassettes were equipped with polycarbonate filters (pore size 0.4 μm) (Nuclepore Corp, Pleasanton, California, USA) for inhalable dust sampling. For the respiratory fraction cellulose filters (pore size 8 μm) (Millipore, Sundbyberg, Sweden) were used for the gravimetric analysis and polycarbonate filters (Nuclepore Corp; pore size 0.4 μm) for estimation of endotoxin concentrations. Sampling was performed by portable pumps at airflow of 1.9 to 2 l/min. The airflow was measured before and after the sampling was completed and an average value of the airflow was used for calculation of sampled air volume. The filters were conditioned 24 h prior weighing, using a Mettler® ME22 balance (Mettler, Greisensee, Switzerland) and reference filters. The filters used for endotoxin analysis (polycarbonate) were extracted by rotation in 10 ml of endotoxin free water for 60 min. The extracts were centrifuged for 10 min at 1000*g* and the supernatant were frozen at −70 °C for later analysis with a chromogen version of *Limulus amebocyte* lysate assay (QCL-1000, Endotoxin, BioWhittaker, Walkersville, USA, with *Escherichia coli* 0111:B4 as standard).

Registration of symptoms

In the morning, prior to the exposure, the subjects filled in a questionnaire regarding symptoms (headache, chills, mental fatigue, muscle pain and nausea). This was repeated 8 h after the start of the exposure. The symptoms were graded according to a visual analogue scale (VAS) ranging from 0 to 100 mm.

Lung function and bronchial responsiveness

Lung function was measured with a wedge-spirometer (Vitalograph®, Buckingham, UK) according to the American Thoracic Society criteria.[16] The highest of three measurements of FEV_1 within 10% and the highest of three slow and three forced vital capacity (VC) manoeuvres were chosen as baseline values. Local reference values were used.[17,18] PEF was measured using a peak flow meter (Mini-Wright, Clement Clarke International Ltd, London, UK).

Bronchial responsiveness was assessed by a methacholine challenge, performed with inhalation

of diluent followed by inhalation of increasing doses of methacholine, starting at 0.5 mg/ml, with doubling concentration steps up to 32 mg/ml.[19] The result is expressed as the cumulative dose causing a 20% decrease in FEV_1 ($PD_{20}FEV_1$). Subjects, who had not attained a 20% decrease in FEV_1 when the maximum dose (12.75 mg) was reached, were assigned a value (15 mg) for the statistical analysis.

Exhaled nitric oxide

Exhaled NO were determined using single-breath exhalations.[20–22] To decrease contamination from the oral cavity, mouthwash with sodium bicarbonate (10%) in 1 min preceded the measurement procedure.[23] The subjects inhaled NO-free air via a mouthpiece to total lung capacity, followed immediately by full exhalation, with a flow-rate of about 100 ml/s, through the mouthpiece into the apparatus. During exhalation, an excess pressure was created in the oral cavity, which ensures closure of the velum and prevents contamination of the sample with nasal air. NO was measured with chemiluminescence after reaction with ozone (Aerocrine NO-system type EBA:1, Aerocrine AB, Stockholm, Sweden). The mean of three measurements was used for evaluation.

NAL

NAL was performed using a procedure described by Bascom and Pipkorn,[24,25] with minor modifications.[7] Briefly, 5 ml sterile 0.9% NaCl was instilled into one nostril using a needle-less syringe. After 10 s the fluid was expelled into a plastic cup. The procedure was repeated in the other nostril and the lavage samples were pooled. The cell concentration was counted in a Bürker chamber. Cytospin-prepared slides were stained with Hemacolour (Mallinckrodt Baker B.V., Deventer, Holland) stain and 300 cells were counted for cell differentials. Less than 100 cells were considered as too few cells to make an accurate differential count.

Peripheral blood

A total and differential white blood cell count of peripheral blood was determined by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA). Blood was collected into EDTA and incubated with cell surface markers CD45/CD14 (LeucoGate™, Becton Dickinson) in TRUECOUNT™ absolute count tubes (Becton Dickinson) for 15 min at room temperature in darkness. The samples were then haemolyszed using FACS™ Lysing Solution (Becton Dickinson) and incubated for 15 min at room temperature in darkness and finally analysed by flow cytometry.

Cytokine analyses in NAL and blood

Interleukin-6 (IL-6) in peripheral blood and NAL fluid and IL-8 in NAL fluid were determined using a specific ELISA validated in our laboratory[26] using commercially available antibody pairs (R&D systems, Europe, Abingdon, UK). The lower detection limit was 3 ng/l for IL-6 and 50 ng/l for IL-8. For duplicate samples an intra-assay coefficient of variation (CV) of $<10\%$ and an inter-assay CV of $<20\%$ was accepted. If the levels were below the detection limit, the samples were assigned a value (below the limit) for statistical analysis.

Measurements of LTB_4, LTE_4 and 9α, 11β-PGF_2 in NAL and urine

Analysis of 9α, 11β-PGF_2 and LTE_4 in the urine, as well as LTE_4 and LTB_4 in NAL fluid, were performed without prior purification with enzyme immunoassays (Cayman Chemical, Ann Arbor, MI, USA) using rabbit polyclonal anti-sera and acetylcholinesterase-linked tracers as described previously[27,28] with the following modifications. The LTE_4 assay was performed with a cysteinyl-leukotriene rabbit polyclonal anti-serum with cross-reactivity to LTC_4 (100%), LTD_4 (100%) and LTE_4 (67%). The standard curve was set up with LTE_4 as unlabelled ligand and LTE_4 linked to acetylcholinesterase was used as tracer. Creatinine was determined in all urine samples using a commercial available colorimetric assay (Sigma Chemical Company, St Louis, MO, USA). The results are expressed as ng per mmol of creatinine or ng/mL for urine and NAL samples, respectively. Regarding urine samples, the mean value of two samples obtained in the afternoon on the pre-exposure day were compared with the mean of two samples obtained at the same time points on the exposure day.

Statistics

Results are presented as median value (25th to 75th percentiles) except for lung function data, oral temperature and urinary levels of LTE_4 and 9α, 11β-PGF_2, that are presented as mean value (95% confidence interval). For all parameters except LTB_4 in NAL fluid, there was no statistically significant difference between pre-medication and pre-exposure values. Therefore, Wilcoxon's signed rank test was used for paired comparisons (pre- and

post-exposure) and differences between groups were assessed by Mann–Whitney *U*-test. Student's *t*-test was used for analysis of lung function data, oral temperature and symptom scores. Correlations were estimated by Spearman Rank correlation test. A *P*-value <0.05 was considered significant.

Results

Exposure measurements

The inhalable and respirable dust levels during the exposure were 10 (9.7–16) mg/m^3 and 0.73 (0.54–0.83) mg/m^3, respectively. The corresponding endotoxin concentration in inhalable dust and respirable dust was 580 (360–830) and 39 (34–40) ng/m^3.

Symptoms

The oral temperature in the morning prior to exposure was 36.4 (36.0–36.8) °C in zileuton treated subjects and 37.1 (36.5–37.7) °C in the placebo group (not statistically significant (ns)) and increased after the exposure by 0.73 (0.10–1.35) °C ($P<0.05$) in the zileuton group and 0.41 (0.20–0.62) °C ($P<0.01$) in the placebo group (ns between groups). Chills increased after the exposure in the zileuton group ($P<0.01$) but not in the placebo group whereas headache increased in both groups ($P<0.05$).

Lung function and bronchial responsiveness

FEV_1 decreased slightly but significantly in both groups following exposure, 7.2% in the placebo group and 4.2% in the zileuton group compared with pre-exposure values (Table 1). Vital capacity decreased significantly in the placebo group (2.8%, $P<0.05$), but not in the zileuton group (1.8%). The bronchial responsiveness to methacholine increased in all subjects by 2.7 (1.6–4.2) and 1.9 (1.5–2.5) doubling concentration steps in the placebo and zileuton group, respectively (Fig. 2). There were no significant differences in the change of lung function or bronchial responsiveness between the groups.

Leukotrienes in NAL and urine

The exposure increased urinary LTE_4 levels in the placebo group ($P<0.05$), but not in the zileuton group (Fig. 3A). There was however, not a statistically significant difference between the groups. The medication did not influence baseline LTE_4 excretion in urine (data not shown).

The LTB_4 level in NAL decreased during treatment with zileuton ($P<0.05$) and remained unchanged after the exposure. In the placebo group, the LTB_4 levels increased after the exposure ($P<0.05$) with a significant difference compared to the zileuton group ($P<0.05$, Fig. 4C, left panel).

Neither the medication with zileuton nor the exposure affected the concentration of LTE_4 in NAL fluid (Fig. 4C, right panel).

9α, 11β-PGF_2 in urine

Medication did not influence basal levels of 9α, 11β-PGF_2 in urine (data not shown). The excretion of 9α, 11β-PGF_2 in urine increased in the placebo group after the exposure (Fig. 3B, $P<0.01$), but not in the group given zileuton ($P<0.05$ between groups).

Table 1 Lung function before and after exposure to organic dust (mean (95% confidence interval)).

	Pre-exposure	Post-exposure	*P*-value
Placebo			
FEV_1 (l)	4.18 (3.74–4.6-2)	3.88 (3.45–4.32)	<0.001
VC (l)	5.06 (4.48–5.65)	4.92 (4.39–5.44)	<0.05
FEV_1/VC (%)	83 (80–86)	79 (75–83)	<0.05
PEF (l/min)	592 (545–638)	505 (457–554)	<0.001
Zileuton			
FEV_1 (l)	3.77 (3.24–4.30)	3.61 (3.10–4.13)	<0.01
VC (l)	4.38 (3.83–4.94)	4.30 (3.74–4.86)	ns
FEV_1/VC (%)	86 (82–90)	84 (80–88)	<0.01
PEF (l/min)	544 (484–603)	495 (433–556)	<0.001

There were no statistical significant differences between the groups, regarding pre- and post-exposure values. Basal FEV_1 was 97% and 95% of predicted and basal VC was 95% and 91% of predicted in the placebo and zileuton group, respectively.

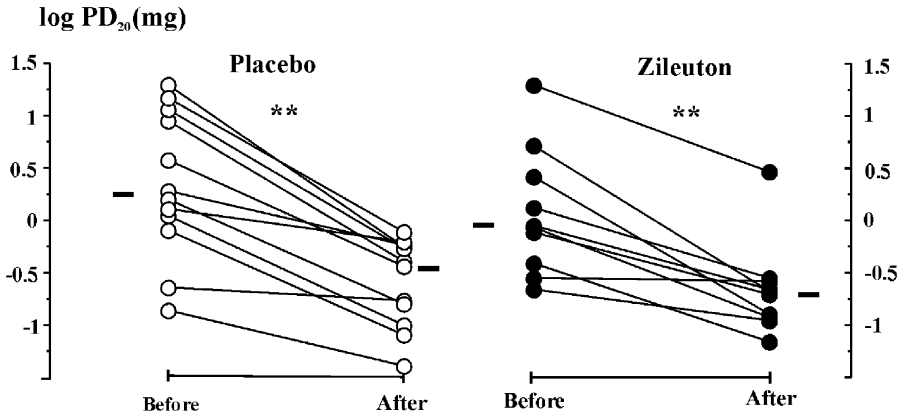

Figure 2 Bronchial responsiveness to methacholine before and after exposure in a swine confinement house in 12 healthy subjects treated with placebo and 10 healthy subjects treated with zileuton. The exposure induced a decrease in $PD_{20}FEV_1$ in all subjects. Horizontal lines indicate median values. $^{**}P<0.01$ for pre- and post-exposure comparisons. Data from one subject was excluded from the statistical analysis due to a technical error during the methacholine provocation test.

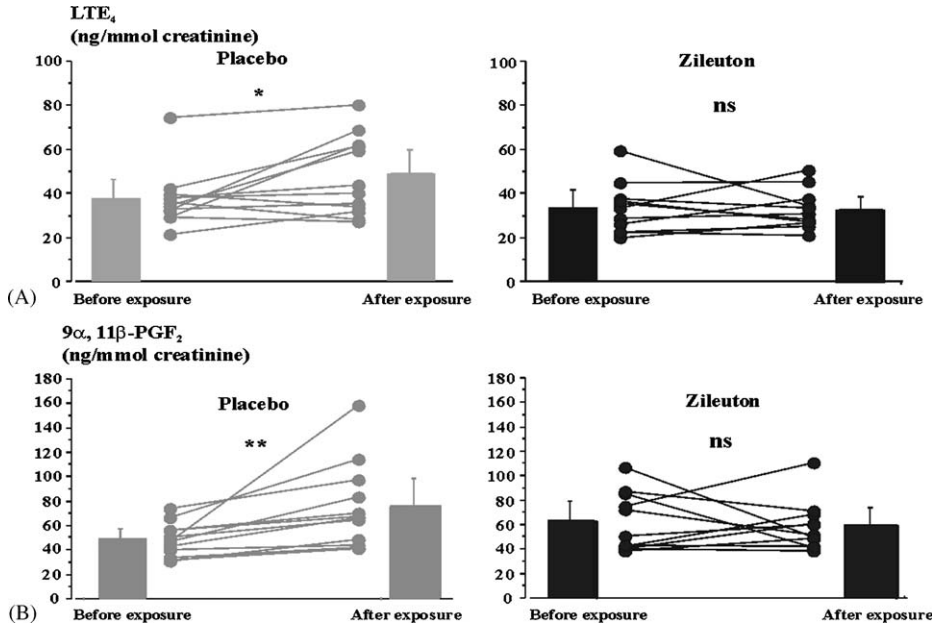

Figure 3 LTE_4 (A) and 9, 11-PGF_2 (B) and in urine samples collected from 11 subjects treated with zileuton and 12 subjects treated with corresponding placebo. Pre-exposure values are the mean value of two samples collected in the afternoon the day before exposure. Post-exposure values are the mean of two samples collected in the afternoon after the exposure. There was a significant difference between the groups regarding the change in concentration of 9, 11-PGF_2 ($P<0.05$) but not for LTE_4. Mean (95% confidence interval) and individual values. *, $P<0.05$; **, $P<0.01$ for pre- and post-exposure comparisons.

Exhaled nitric oxide

Exhaled nitric oxide was more than doubled in both groups after exposure ($P<0.01$) and increased from 6.7 (4.3–11) to 14 (8.6–20) ppb in the zileuton group and from 6.6 (5.6–8.3) to 14 (12–19) ppb in the placebo group. There was no significant difference between the groups.

NAL

The cellular composition was not influenced by the medication. Exposure caused a significant increase of total cells in both groups ($P<0.01$, Fig. 4A) with no difference between the groups. The increase was mainly due to elevated numbers of neutrophilic granulocytes (data not shown). No changes were

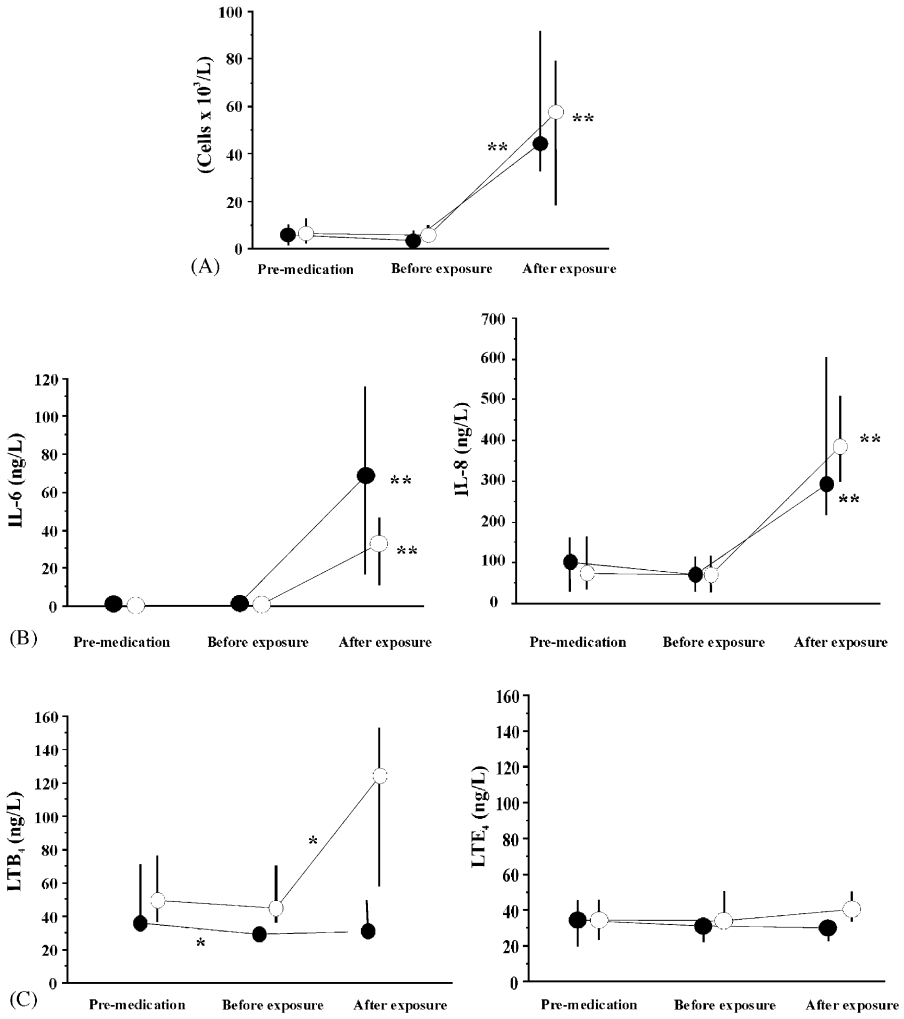

Figure 4 Total cell concentration (A), the concentration of IL-6 and IL-8 (B) and the concentration of LTB_4 and $LTEC_4$ (C), in NAL fluid before and after five days of medication and after exposure to swine dust. Open circles represent placebo ($n = 12$) and filled circles represent zileuton ($n = 11$) treatment. Significant increases were observed in total cell concentration and cytokine levels in both groups following exposure. LTB_4 levels decreased after 5 days of zileuton treatment, and were not further influenced by the exposure. The placebo group demonstrated significantly elevated levels of LTB_4 after exposure. Levels of LTE_4 were not affected by drug pre-treatment or exposure. There were no significant differences between the groups except for LTB_4 ($P<0.05$). Median and interquartile range are presented. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ for pre- and post-exposure comparisons, # indicates significant differences between the groups.

observed concerning epithelial cells, monocytes, lymphocytes, or eosinophilic and basophilic granulocytes. No correlation between increase in neutrophilic granulocytes and LTB_4 was observed in the placebo group ($\rho = -0.04$, $P = 0.89$).

The concentration of IL-6 in NAL fluid increased in both groups following exposure to swine house dust ($P<0.01$) with no statistically significant difference between the two groups (Fig. 4B). The exposure induced a significant increase in IL-8 concentration in NAL fluid in both groups (Fig. 4B), with no difference related to treatment. There was no correlation between the increase in neutrophilic granulocytes and IL-8 ($\rho = 0.37$, $P = 0.27$ for zileuton; $\rho = -0.04$, $P = 0.89$ for placebo).

Peripheral blood

Treatment with zileuton or placebo did not influence the cellular composition in the peripheral blood. After exposure the concentration of neutrophilic granulocytes and monocytes increased in both groups ($P<0.001$, Fig. 5A). The increase in neutrophils and monocytes was greater in zileuton-treated subjects ($P<0.05$).

IL-6 increased in both groups following exposure (Fig. 5B, $P<0.05$ for placebo, $P<0.01$ for zileuton), significantly more in the zileuton group ($P<0.001$).

Discussion

The purpose of the study was to assess the significance of leukotrienes in the mechanism underlying the increase in bronchial responsiveness induced by exposure in a swine confinement facility. There are observations suggesting a role for leukotrienes in increased bronchial responsiveness to other stimuli. For example, the cysLT$_1$ receptor antagonists zafirlukast and montelukast have both been found to reduce allergen-induced airway hyperreactivity in atopic asthmatic subjects.[11,29] Furthermore, when zileuton (600 mg qid) was given as an add-on treatment to glucocorticosteroids, Dahlén et al. reported a significantly reduced bronchial responsiveness to histamine after 6 weeks treatment in subjects with aspirin-intolerant asthma.[30] It has also been reported that zileuton inhibited the response to cold dry air challenge in asthmatic subjects in a fashion suggesting a general effect on bronchial responsiveness.[10] These effects of zileuton may support a role for cysteinyl-leukotrienes, but may also indicate participation of LTB$_4$ in bronchial inflammation induced by different stimuli.

In the present study of healthy, non-allergic subjects, we were, however, not able to demonstrate any influence of zileuton treatment on the

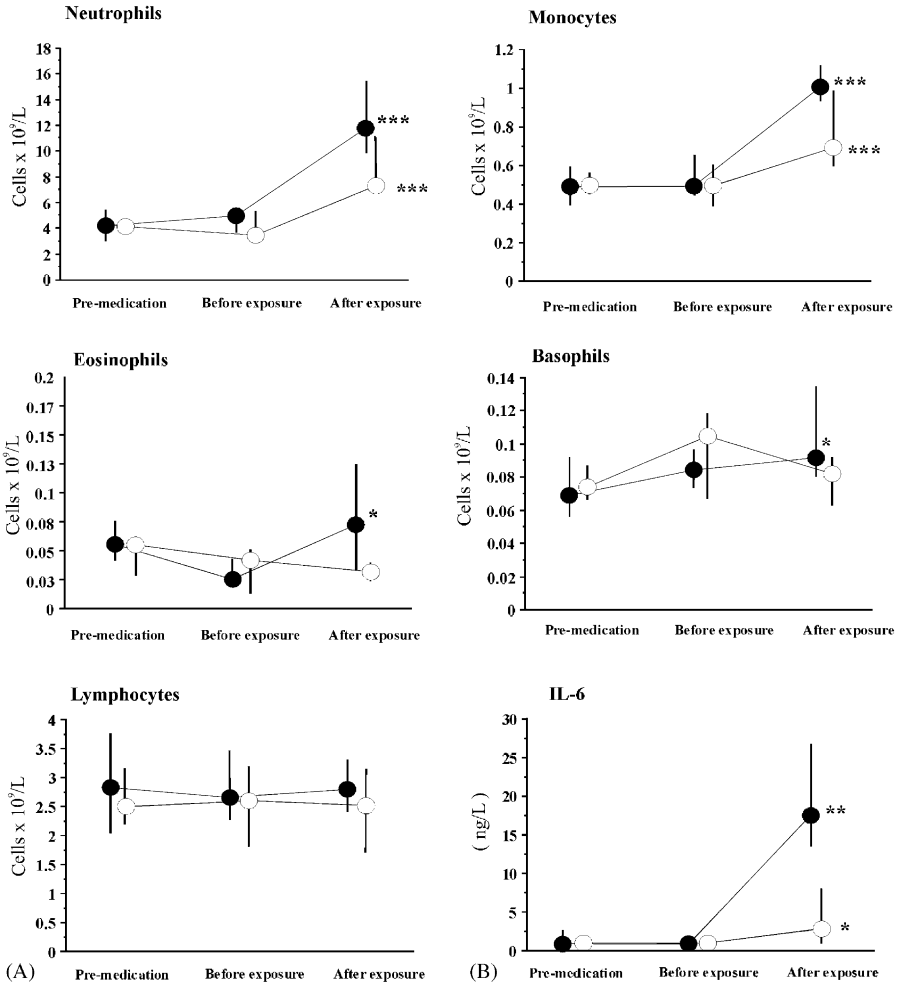

Figure 5 Cellular reactions (A) and IL-6 (B) in peripheral blood before, and during medication and after exposure to swine house dust. Open circles represent placebo ($n = 12$) and filled circles represent zileuton ($n = 11$) treatment. The increase in concentration of neutrophils and monocytes differed significantly between the groups ($P<0.05$). IL-6 increased significantly more in the zileuton group than in the placebo group ($P<0.001$). Open circles represent placebo and filled zileuton. Median and interquartile range are presented. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ for pre- and post-exposure comparisons, # indicates significant differences between the groups.

increased bronchial responsiveness induced by the swine dust exposure. We cannot conclude whether our finding implicate that leukotrienes are not involved in the mechanisms underlying increased bronchial responsiveness in this particular model, or if the treatment period (5 days) and/or the dose (600 mg qid) were inadequate to produce major inhibition of leukotriene formation. Measurements of urinary LTE_4 excretion would seem to support the latter alternative, as there was no significant difference in neither post-medication nor post-exposure urinary LTE_4 between placebo and active treatment. However, as urinary LTE_4 increased significantly post-exposure only in the placebo group but not in the zileuton group, the findings suggests a partial inhibitory effect on cys-LTs formation.

We found a post-exposure increase in LTB_4 in NAL fluid in the placebo group, but not in subjects treated with zileuton. The finding supports a significant inhibitory effect of zileuton on local leukotriene biosynthesis in the nasal cavity. The results may indicate that local LTB_4 production is inhibited by zileuton more easily than is whole-body formation of cys-LTs, as reflected by the measurements of urinary excretion of LTE_4. This view is supported by a previous study of Hui et al. in patients with atopic asthma. They found that a single dose of zileuton (800 mg) almost totally blocked the ex vivo formation of LTB_4 in whole blood, whereas allergen-induced urinary excretion of LTE_4 was only reduced to about 50% in the same subjects, in association with a non-significant effect on the bronchoconstriction.[31] In line with this, in our study zileuton only caused a threshold inhibition of systemic leukotriene biosynthesis, but had a clear-cut effect on local production in particular of LTB_4. A complete inhibition of leukotrien formation is needed before it can be concluded that leukotrienes are not involved in the increased bronchial responsiveness induced by exposure to swine house dust.

The dose of zileuton that was used in the study is established to have clinical anti-asthmatic effect. It is however known, that exposure to a swine house environment represents a very strong pro-inflammatory stimulus. For example, a more than 70-fold increase of the concentration of neutrophilic granulocytes is demonstrated in bronchoalveolar lavage fluid from healthy subjects 24 h after 3 h of exposure in a swine confinement building.[6,7] We therefore speculate that the degree of enzyme inhibition needs to be larger in this particular model than in chronic asthma. It is established that the enzymes in the leukotriene pathway may be up-regulated.[32] Therefore, it is not unlikely that the enzymes in the biosynthetic pathway may become up-regulated during the course of this exposure to many aggressive factors such as endotoxin and bacterial peptidoglycans.[7,33] Furthermore, little is known about how large inhibition of, for example, urinary LTE_4 that is required for clinical efficacy. In studies where clinical improvements have been documented, the inhibition of urinary LTE_4 has been about 50%.[30,34]

The present study confirmed our previous indications that mast cells are activated following the exposure as increased urinary levels of the PGD_2 metabolite 9α, 11β-PGF_2 were detected.[3] The post-exposure urinary excretion of 9α, 11β-PGF_2 was lower in the group treated with zileuton. Although the limited effect of zileuton on systemic leukotriene production calls for cautious interpretation of the finding, the decreased levels of 9α, 11β-PGF_2 were observed both within the group and between the placebo and zileuton group. As zileuton has no direct effect on the cyclooxygenase,[35] this raises the hypothesis that 5-lipoxygenase products somehow are involved in mast cell activation or recruitment during this particular reaction. This may be a general mechanism as release of tryptase has been reported to be inhibited by zileuton in NALs collected after aspirin challenge of aspirin-intolerant asthmatics.[36]

Further support for mast cell activation following exposure in this farming environment comes from a previous study where pre-medication with sodium cromoglycate attenuated the inflammatory response after exposure to swine house dust in healthy subjects, with reduced post-exposure increases of neutrophils, TNF-α and IL-6 levels in bronchoalveolar lavage fluid.[37] The mechanism of action of sodium cromoglycate includes stabilisation of the mast cell and inhibition of mediator release.[38] Since TNF-α is a typical mast cell cytokine[39] and the TNF-α response was almost abolished by cromoglycate treatment,[37] these results strengthen the hypothesis that mast cells are involved in the inflammatory response after exposure to swine house dust. Despite these inhibitory effects of cromoglycate on cytokine generation in this particular model, cromoglycate did not significantly inhibit the increase in bronchial responsiveness following the exposure.[37] As cromoglycate and zileuton otherwise both are effective against many common triggers of asthma, we conclude that pharmacological intervention to modify the increased bronchial responsiveness following swine house exposure may require fairly aggressive treatments. In order to study underlying mechanisms in a broader perspective, it would be interesting to combine different asthma drugs,

which have been tested separately in this model, in order to see if additive or synergistic effects are obtained.

In the present study, post-exposure blood concentration of neutrophils and monocytes was significantly higher in subjects treated with zileuton compared to placebo. The symptom scores for chills increased significantly only in zileuton-treated subjects and this could possibly have been related to the higher serum levels of the pro-inflammatory cytokine IL-6, which is involved in the induction of fever reactions.[40] The explanation to these findings is not clear, but suggests that leukotrienes and/or other 5-lipoxygenase products might have anti-inflammatory properties as well. One such group of compounds is the lipoxins, which have inhibitory effects on neutrophil activation.[41] It has been shown that lipoxin A_4 inhibits the IL-1β-induced IL-6 and IL-8 response in human synovial fibroblasts.[42] The possibility that the 5-lipoxygenase in the current exposure model induces the formation of both pro- and anti-inflammatory endogenous substances would in addition make the effect of 5-lipoxygenase inhibition more complex to evaluate, and may contribute to the limited effects we observed with zileuton on the main endpoints. One explanation could be that lipoxins on the one hand, and the cysteinyl-leukotrienes on the other, have opposing actions and that selective intervention with a $cysLT_1$ receptor antagonist is required to affect bronchial responsiveness.

Increased levels of exhaled NO after exposure using this particular model, have been demonstrated in an earlier study,[43] and were also confirmed in the present study. In fact, the NO levels were increased to the same magnitude (two-fold) as has been measured in moderate asthma using a similar method for measurements.[44] The NO response was not modified by the treatment with zileuton. As discussed, this may either reflect insufficient inhibition of leukotrienes or that leukotrienes are not directly involved in the NO formation, which is indicated by a study where asthmatic subjects displayed no change in NO levels after inhalation of LTE_4.[45] On the other hand, there are studies suggesting direct relations between leukotrienes and airway generation of NO. For example, 2 weeks of treatment with montelukast reduced the exhaled NO in asthmatic children by 20%[46] and in adult asthmatics pranlukast inhibited the increase in exhaled NO when the use of high-dose corticosteroid was reduced by half.[47] The present study included healthy non-asthmatic subjects and the mechanisms responsible for induction of NO synthesis might also differ between normal and asthmatic subjects.

In summary, we failed to achieve significant inhibition of bronchial responsiveness and urinary leukotriene excretion after exposure in a swine confinement house following 5 days pre-treatment with the clinically used dose of the 5-lipoxygenase inhibitor zileuton. One conclusion from this finding is that the inflammatory response in this specific model is different, complex and more difficult to inhibit than common asthmatic reactions.

Moreover, the study reinforces the concept that swine house dust induced airway inflammation is fundamentally different from common asthmatic reactions. The observed failure of zileuton, an established anti-asthmatic drug, to inhibit the reaction, adds yet another asthma medication that is ineffective in this model. If this profile of drug-resistant bronchial hyperresponsiveness relates to the induced neutrophilic airway inflammation, the model may have relevance also for studies of mechanisms in chronic bronchitis and COPD.

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